

**WHAT IS CLAIMED IS:**

- 1           1. An integrated plasmid comprising a biotin synthase  
2 gene, an assistant DNA sequence for the integration of said  
3 plasmid into a host genome, a promoter sequence, and a  
4 selection marker.
- 1           2. The integrated plasmid as claimed in claim 1,  
2 wherein the biotin synthase gene is derived from  
3 *Saccharomyces cerevisiae* or *Candida utilis*.
- 1           3. The integrated plasmid as claimed in claim 2,  
2 wherein the biotin synthase gene of *Candida utilis* comprises  
3 the nucleotide sequence of SEQ ID NO: 1.
- 4           4. The integrated plasmid as claimed in claim 1,  
5 wherein the assistant DNA sequence is a *Candida utilis*  
6 fragment selected from the group consisting of NsiI-BamHI  
7 18s rDNA, URA3 DNA, and HIS3 DNA.
- 1           5. The integrated plasmid as claimed in claim 1,  
2 wherein the selection marker is a cycloheximide-resistant  
3 gene.
- 1           6. The integrated plasmid as claimed in claim 1,  
2 wherein the promoter sequence is selected from the group  
3 consisting of pL41 promoter of *Candida utilis* and pADH1  
4 promoter of *Saccharomyces cerevisiae*.

1           7. The integrated plasmid as claimed in claim 1,  
2 wherein the integrated plasmid is selected from the group  
3 consisting of:

4           (a) pMCC21 (having the configuration of restriction  
5 sites in FIG. 6);

6           (b) pMCC31S (having the configuration of restriction  
7 sites in FIG. 8);

8           (c) pMCC32H (having the configuration of restriction  
9 sites in FIG. 9);

10          (d) pMCC33U (having the configuration of restriction  
11 sites in FIG. 10);

12          (e) pMCC35U (having the configuration of restriction  
13 sites in FIG. 11);

14          (f) pMCC36H (having the configuration of restriction  
15 sites in FIG. 12); and

16          (g) pMCC38S (having the configuration of restriction  
17 sites in FIG. 13).

1           8. A method for preparing a yeast with high biotin-  
2 productivity, comprising the steps of:

3           constructing an integrated plasmid comprising a biotin  
4 synthase gene, an assistant DNA sequence for the integration  
5 of said plasmid into a host genome, a promoter sequence, and  
6 a selection marker;

7           linearizing said integrated plasmid;

8           transforming said linearized integrated plasmid into a  
9 yeast; and

10          recombining the biotin synthase gene with the yeast  
11 genome.

1           9. The method as claimed in claim 8, wherein the biotin  
2     synthase gene is derived from *Saccharomyces cerevisiae* or  
3     *Candida utilis*.

1           10. The method as claimed in claim 9, wherein the  
2     biotin synthase gene of *Candida utilis* comprises the  
3     nucleotide sequence of SEQ ID NO: 1.

1           11. The method as claimed in claim 8, wherein the  
2     assistant DNA sequence is a *Candida utilis* fragment selected  
3     from the group consisting of NsiI-BamHI 18s, rDNA, URA3 DNA,  
4     and HIS3 DNA.

1           12. The method as claimed in claim 8, wherein the  
2     selection marker is a cycloheximide-resistant gene.

1           13. The method as claimed in claim 8, wherein the  
2     promoter sequence is selected from the group consisting of  
3     pL41 promoter of *Candida utilis* and pADH1 promoter of  
4     *Saccharomyces cerevisiae*.

1           14. The method as claimed in claim 8, wherein the  
2     prepared yeast with high biotin-productivity is useful as  
3     feed additives, food additives, or cosmetics.

1           15. A method for producing biotin, comprising:  
2     providing the yeast with high biotin-productivity of  
3     claim 8; and  
4     culturing said yeast in a nutrient medium, and  
5     recovering biotin from the culture broth.

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1           16. The method as claimed in claim 15, wherein the  
2   recovered biotin is useful as feed additives, food additives,  
3   or cosmetics.